What is isothermal amplification?

The Polymerase Chain Reaction (PCR) is a well-known approach for amplifying a specific DNA or RNA (e.g., PCR) sequence. PCR involves the repetitive cycling of a reaction cocktail between different temperatures to achieve amplification. As routine as PCR is in the molecular biology and molecular diagnostic laboratory, there are other methods of isothermal nucleic acid amplification.

These alternative approaches often do not require changing the reaction temperature and are referred to as isothermal amplification protocols. Isothermal amplification protocols are varied and have different advantages. In general, isothermal techniques are extremely fast and do not require thermocyclers, making them particularly well-suited for field applications and point-of-care molecular diagnostics assays.

Advantages

• Fast
• Minimal equipment required
• Robust reactions in the presence of inhibitors
• Options for amplified optical detection

Optimization tips for LAMP

• Use LAMP primer design software (e.g., NEB-LAMP Primer Design Tool, https://www.neb.com) to select 2–3 sets for each target and optimize performance in a LAMP assay.
• Include loop primers for faster reactions.
• Use high complex reaction buffer (e.g., NEB #M0291) for a 1× minimal formulation for best reactions.
• Optimize the reaction temperature (60–70°C for Bst 2.0/3.0, 55–59°C for Bst 2.0/3.0 LF) and ANTP (1–1.4 mM) concentrations for best reactions.

Interested in learning how NEB scientists are using isothermal amplification? Visit www.neb.com/sampling to find videos, protocols and recent publications, including a publication from NEB scientists describing pt-sensitive isothermal detection.

Featured products for isothermal amplification

WarmStart® Multi-Purpose LAMP/RT-LAMP 2X Master Mix (with UDG) (NEB #M1708)

Loop-Mediated Isothermal Amplification (LAMP) is a commonly used technique for nucleic acid detection. NEB’s WarmStart LAMP products provide a simple, one-step solution for DNA or RNA targets. The WarmStart Multi-Purpose LAMP/RT-LAMP 2X Master Mix (with UDG) is fully buffered and compatible with different template types, enabling multiple detection methods including turbidity detection, real-time fluorescence detection, and end-point visualizations such as chromogenic detection via a metal indicator (e.g., hydroxynapthol blue). It features Bst 2.0 Warmstart DNA Polymerase and WarmStart RTx Reverse Transcriptase, both in-house-designed enzymes for improved performance in LAMP reactions. For real-time fluorescence detection, the master mix is available as a kit (NEB #M1710) that includes NEB LAMP Fluorescent Dye.

WarmStart RTx Reverse Transcriptase

WarmStart RTx Reverse Transcriptase (NEB #M0380) is an in vitro RNA-directed DNA polymerase coupled with a reversibly-bound aptamer that inhibits RTx activity below 40°C. This enzyme is synthesized as a complementary DNA strand initiating from a primer using RNA (DNA synthesis) or single-stranded DNA as a template. RTx is a robust enzyme for DNA detection in amplification reactions and is particularly well-suited for use in LAMP. The WarmStart property enables high throughput applications, robust temperatures, and increases the consistency and specificity of amplification reactions’ RTx inhibitors’ kinetic activity.

WarmStart control of WarmStart RTx

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Not sure which product will work best for your experiment? NEB offers a selection of 8x DNA Polymerase-based products for isothermal amplification. Use this chart to determine which product will work best for your needs.

<table>
<thead>
<tr>
<th>LAMP/RT-LAMP Reaction</th>
<th>DNA Polymerase 2.0</th>
<th>DNA Polymerase 3.0</th>
<th>DNA Polymerase, Large Fragment</th>
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<tbody>
<tr>
<td>AMV reverse transcriptase</td>
<td>*</td>
<td>*</td>
<td>N/A</td>
</tr>
<tr>
<td>RT-LAMP</td>
<td>**</td>
<td>**</td>
<td>**</td>
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<tr>
<td>Nick translation reactions at elevated temperatures</td>
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<tr>
<td>Single-strand exponential reactions, coupled with a reversibly-bound aptamer that inhibits RTx activity below 40°C</td>
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<tr>
<td>Improved LAMP, SDA, and other amplification reactions</td>
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<tr>
<td>Conserved room temperature and high-temperature amplification, coupled with a reversibly-bound aptamer that inhibits RTx activity below 40°C</td>
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<tr>
<td>Engineered and tested to work readily with multi-stranded DNA targets</td>
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<td>**</td>
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<tr>
<td>High reverse transcription activity at 54°C</td>
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</tbody>
</table>

* Not applicable to this application
** Works with this particular application
N/A Not applicable or inapplicable
** Best reaction conditions are 50°C for DNA Polymerase 3.0, 55°C for DNA Polymerase, Large Fragment

Did you know that many of these products can be purchased in large volume? Contact customercare@neb.com to find out more.
Examples of isothermal technologies

Loop-mediated Isothermal Amplification (LAMP & RT-LAMP)

LAMP uses 4-6 primers recognizing 6-8 distinct regions of target DNA for highly specific amplification reactions. A strand-displacing DNA polymerase initiates synthesis and 2 typically designed primer pairs act to generate subsequent rounds of amplification through extension on the 3’ and 5’ termini of nascent DNA products. LAMP products are very low (< 20 kb) and formed from numerous repeat of the 150-200 bp target sequence, connected with single-stranded loop regions in long concatamers. These products are not typically appropriate for downstream manipulation, but target amplification is necessary to ensure that contaminating DNA is not amplified. The reaction is monitored by using interactions of probes, fluor and agarose gel detection as all directly compatible with LAMP reaction. Instruments for LAMP typically require controlled heating to the desired reaction temperature and, where needed, real-time fluorescence for quantitative measurement.

Nucleic Acid Sequenced Based Amplification (NASBA)

NASBA and Transcription Mediated Amplification (TMA) are similar isothermal amplification techniques that proceed through RNA. Primers are designed to target a region of interest, but in contrast with LAMP, one primer includes an RNA hairpin sequence for T7 RNA polymerase at the 3’ end. This enables production of single-stranded RNA, which is reverse transcribed into complementary DNA by reverse transcription included in the reaction. The RNA in the DNA-RNA hybrid is destroyed by RNase H allowing an exonuclease process in NASBA, or by an RNase H- or RT in TMA and NASBA is produced by the RT. Then amplification is triggered by an RNA or RT/ RNA polymerase and exponential amplification results.

Nicking Enzyme Amplification Reaction (NEAR)

NEAR employs a strand-displacing DNA polymerase containing a nicking enzyme by nicking targets, rapidly producing many short nucleic acids from the target sequence. This process is sensitive, fast and allows enabling detection of small target amounts in minutes. NEAR is usually used for pathogen detection in clinical and biotechnology applications.

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